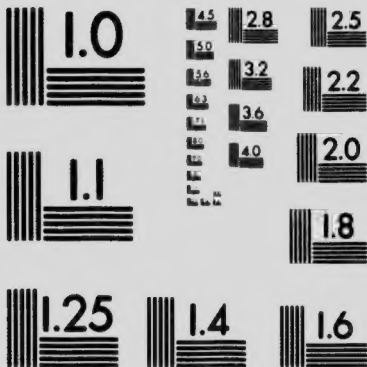




Unibersity of Toronto.

DEPARTMENT OF PATHOLOGICAL CHEMISTRY.



**Elementary Practical
Course.**



**UNIVERSITY PRESS
TORONTO.**

Norman Fount

University of Toronto.

DEPARTMENT OF PATHOLOGICAL CHEMISTRY.



**Elementary Practical
Course.**



**UNIVERSITY PRESS
TORONTO.**

THE EXAMINATION OF URINE.

The composition and concentration of the urine varies from hour to hour during the day. Any sample of urine may often serve for the qualitative detection of sugar, albumin or bile. But it is no use attempting to decide whether more or less than is normal of any constituent is being passed, unless the time is known that it has taken for a given measured quantity of urine to be secreted. Usually it is best to collect the urine for 24 hours, mix it, measure it and do all determinations, even that of the specific gravity, only on such a 24-hours' sample. To obtain this have the bladder emptied at a certain hour and reject this sample, but keep all that is passed till the same hour next day when the bladder is emptied again. A preservative must be added to the first portion kept, a few c.c. of chloroform, or if the reduction of copper solutions is to be tested for, then toluene instead.

The specific gravity of the urine depends on the amount of solids excreted and of water in which they are dissolved; even with the former constant, if the latter varies the sp. gr. will too. The amount of water leaving the kidneys depends on the amount lost by the body by other channels, skin, lungs, or intestines. Usually it may be said that of the total loss of water in 24 hours:

18%	or 1200 c.c.	is lost by way of the kidneys,
32%	or 800 c.c.	" " skin,
16%	or 400 c.c.	" " lungs,
4%	or 100 c.c.	" " intestines.

But the last three are subject to very great variations depending on the external temperature and humidity of the air, the development of heat by muscular and other activities of the body, the depth and frequency of respiration and the action of the bowels. The kidneys have great powers of adaptation and excrete less or more water according as more or less is removed in other ways, and while the average sp. gr. of human urine is 1.016 to 1.020, it may be perfectly normal for it to have any value from 1.005 to 1.030 at least. But a high sp. gr. normally occurs only when the volume is diminished and vice versa, and any departure from this rule is pathological, e.g., in diabetes high sp. gr. in a large volume.

Take the sp. gr. of urine at room temperature and again at 37° C.: note the difference and remember always to cool urine before taking its sp. gr.

Determination of acidity of urine. A standard solution is one of known strength; a normal solution contains 1 gramme equivalent (Mol. Wt. $\times$ 1 gramme) of the substance dissolved in 1 litre: e.g., normal soda 40 g. in 1 l. or 4%, decinormal soda 0.4%. Any volume of normal acids neutralizes an equal volume of normal alkalies: therefore while normal HCl contains 36.5 g. HCl in 1 litre, normal oxalic or sulphuric (dibasic) acids contain $\frac{1}{2}$ Mol. Wt. $\times$ 1 gramme in 1 litre.

Prepare decinormal oxalic acid. Weigh 3.15 g. (48.5 grains) of pure dry oxalic acid $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$, in a small beaker; add about 20 c.c. of distilled water and warm till dissolved. Pour the solution without loss into a 500 c.c. measuring flask, rinse the beaker several times into the flask and fill up to the mark with water. Mix thoroughly by inverting and shaking. Pour into a dry bottle and label 0.1 N oxalic acid.

Prepare decinormal soda. Weigh out 2.5 g. (40 grains) of caustic soda, dissolve and transfer as above into 500 c.c. flask, rinse, fill up and mix. Now standardize the solution by comparison with the 0.1 N oxalic acid. Measure 10 c.c. of the soda solution with a pipette into a beaker or flask, add 2 drops of phenolphthalein (0.5% solution in alcohol) and add 0.1 N oxalic acid from a burette till the pink colour just disappears: note the amount used. Repeat with another 10 c.c. of soda and take the mean of the two results, if they are close. If 10 c.c. exactly of oxalic acid has been used the soda is decinormal; if more than 10 c.c., say 11 c.c., was necessary then every 10 c.c. of soda solution left in the flask will require 1 c.c. of water to be added to it in order to become decinormal. Add the necessary amount of water, mix and take 10 c.c. and titrate again. (If less than 10 c.c. of oxalic acid neutralizes 10 c.c. of soda, say 9 c.c., then label the solution 0.09 N. NaOH.)

The reaction of any aqueous solution is determined by the concentration of hydrogen and hydroxyl ions in the solution. When the number of hydrogen ions in a given volume is exactly equal to the number of hydroxyl ions in the same volume the solution is exactly neutral; if there are more hydrogen ions than hydroxyl the reaction is acid; if less it is alkaline.

Anything which increases the concentration of hydrogen ions diminishes that of the hydroxyl ions in proportion, and vice versa—so that the product obtained by multiplying the concentration of the hydrogen ions by the concentration of the hydroxyl ions is always the same; it has approximately the value $N \times 10^{-14}$, and

therefore in a neutral solution where these two concentrations are equal, each is equal to $N \times 10^{-7}$, or in other words there is in one c.c. of such a solution 0.1 milligram of hydrogen ion present, and 1.7 milligram of hydroxyl ion.

The hydrogen ion concentration is increased by acids, the hydroxyl ion concentration by alkalis, to a degree that varies with the "strength", *i.e.*, the ionic dissociation of the acid or base employed. Strong mineral acids like hydrochloric are nearly completely ionized in dilute aqueous solution, so that 0.1N HCl gives a hydrogen ion concentration which is also very nearly 0.1 N; but weak acids are only slightly ionized and, for instance, acetic acid gives a hydrogen ion concentration that is very much less than 0.1 N (about $N/400$).

Indicators are used for determining the reaction of a solution because the colour of their solutions changes when certain degrees of hydrogen ion concentration are reached: the range of hydrogen ion concentrations within which the colour of a solution of an indicator changes is, if certain conditions are observed, constant for that indicator, but different for the different indicators. This range is illustrated in the case of some of the commonest indicators in the accompanying chart in which the ordinates represent concentration of hydrogen or hydroxyl ion and the vertical lines under the names of the different indicators show the degree of acidity or concentration of hydrogen ions at which the colour of the solution of each indicator begins and ceases to change. At the side of the chart is shown the concentration of hydrogen and hydroxyl ions in pure water and neutral solutions, as well as that of the blood serum which is very constant, of the urine which varies within certain limits and of $N/10$ hydrochloric acid and caustic potash respectively.*

The reaction of normal fresh human urine varies according to diet, but in the case of white races is usually acid. The strong mineral acids HCl and H_2SO_4 occur only as neutral salts in the urine; human urine is never sufficiently acid to react acid to methyl orange or congo paper as would be the case if there were no more free HCl than in a solution of $N \times 10^{-4}$ or $N/10,000$ strength or about $\frac{1}{3}$ of a mg. HCl in 100 c.c.; and 100 c.c. of urine usually contains about $\frac{1}{3}$ of a gramme of HCl neutralized principally as sodium salt so that at any rate less than $1/2000$ or less than 0.05% of the hydrochloric acid in urine

*See Walpole, *Biochemical Journal*, V. 207.

can be free. But of the weak tribasic mineral acid, phosphoric acid, and of the weak organic acid, which are found in urine, considerable quantities may not be completely neutralized, and yet, since these acids are only to a very small extent ionized, the hydrogen ion concentration will be kept low enough not to cause a reaction, with methyl orange. For the complete neutralization of these weak acids the available bases do not usually suffice.

The reaction of the urine is affected by diet because:

(1) The more protein is consumed in the body the more acid the urine will become: for 100 g. of protein contains from 1 to 2 of sulphur which on oxidation gives from 3 to 6 of sulphuric acid (=600 to 1200 c.c. of decinormal acid). The other principal acid product of oxidation in the body, carbonic acid, is removed in the form of gas by the lungs, but the sulphuric acid is all excreted by the kidney. The urine of cats, dogs and carnivora generally is strongly acid.

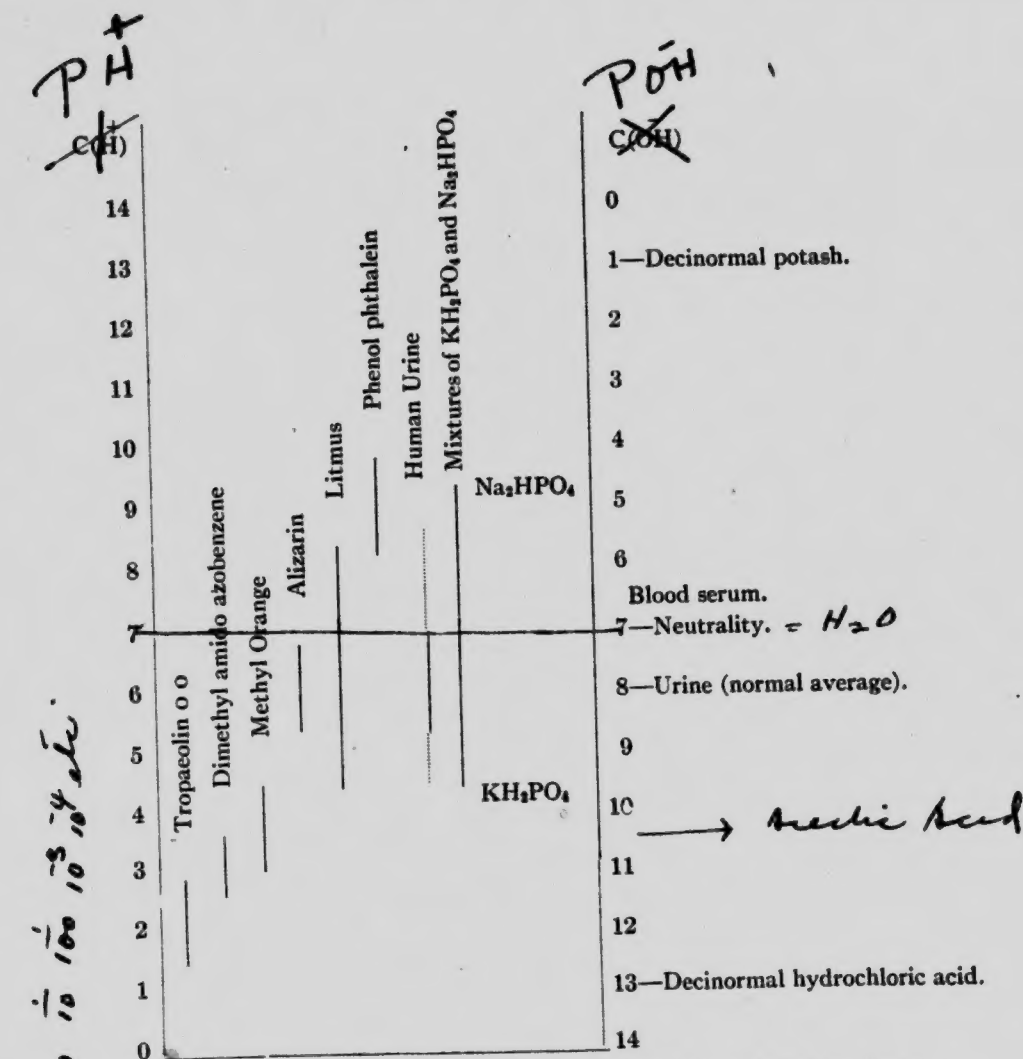
(2) Fruit and vegetables, on the other hand, contain salts, mostly potassium salts, of organic acids. The organic acids are burnt in the body and the CO_2 so formed is exhaled from the lungs, but the bases are left to be removed by the kidney. The urine of horses and other herbivorous animals reacts alkaline and will generally effervesce when acidified.

Human urine approaches more to the one type or to the other according to the diet.

The alkaline tide: when gastric juice is being secreted the separation of free HCl must leave the blood more alkaline and the reaction of the urine becomes accordingly more nearly alkaline; just as when alkaline carbonates or alkaline salts of organic acids are administered and absorbed from the intestine, and as the urine can also be made acid by administering acid phosphates or benzoic or salicylic acids.

The fact that the change in the reaction of the blood, caused by the secretion of gastric juice, at once affects the reaction of the urine excreted by the kidney illustrates the delicacy with which the kidney is adjusted to the performance of one of its most important duties, namely the preservation of the reaction of the blood. Unlike other organs of the body the kidney secretes a fluid the reaction of which may vary from minute to minute and is determined by the necessity of maintaining the reaction of the blood constant.

The reaction of the urine is changed very readily after it has left the renal cells by bacteria in the urinary



The numbers to the right and left of the chart (negative logarithms) indicate the different concentrations of the hydroxyl and hydrogen ions respectively: thus the figure 7 shows the level on the chart at which the concentration of hydrogen ion is $N \times 10^{-7}$; when this is the case the concentration of hydroxyl ion is as the chart shows also $N \times 10^{-7}$; the product of these two concentrations is always the same and therefore the sum of their logarithms, the sum, that is, of the numbers at the two ends of the same line, always is the same, viz., 14.

The concentration of hydrogen ion at the level of the lowest line is $N \times 10^{-0} = N \times 1$, at that of the next it is $N/10$, the next $N/100$, and so on.

The dotted parts of the line for human urine indicate unusual but not necessarily pathological variations of reaction.

passages, or if these are healthy, outside the body. The neutral compound urea is converted by many micro-organisms into ammonium carbonate, giving rise to an alkaline reaction in urine that was acid when passed, and also to an ammoniacal odour.

The reaction of the urine is sometimes said to be amphichroic, or amphoteric, which means that it is such that it turns the colour of red litmus as well as that of blue litmus, in both cases to some intermediate violet tint. As shown in the chart given above the range of hydrogen ion concentrations which correspond to changes in the colour of litmus is a wide one. Solutions with the concentration of H ions somewhere between $N \times 10^{-5}$ and $N \times 10^{-4}$ turn litmus as purely red as it can be turned by even stronger degrees of acidity; and those somewhere between $N 10^{-8}$ and $N 10^{-9}$ turn it as purely blue as stronger degrees of alkalinity. But between these two limits the passage from red to blue is a gradual one through a series of violet tints, becoming successively less red and more blue and each corresponding to some definite intermediate concentration of hydrogen ions. Strictly speaking therefore, as the chart shows, the great majority at any rate of human urines are amphoteric though it may not always be possible to show this by means of the available test papers.

Solutions of KH_2PO_4 and of Na_2HPO_4 are given out. Note the reaction of each of these to litmus and congo papers, to methyl orange, Töpfer's reagent (dimethyl-aminoazobenzene) and to phenolphthalein. Add to 5 c.c. of the acid phosphate some of the alkaline phosphate 1 c.c. at a time till a mixture is obtained which shows the amphichroic reaction; note that this mixture reacts like normal urine, neither acid to methyl orange nor alkaline to phenolphthalein.

The urine may be titrated to methyl orange and in this way compared with a solution such as the acid phosphate solution which gives the methyl orange reaction. To do this put into each of two flasks 10 c.c. of urine, 25 c.c. of water and 2 drops of a solution of methyl orange (0.25% in water). Run decinormal acid from a burette into one of the flasks, having the other standing by for comparison, and note when the colour changes.

Usually, however, the urine is titrated as follows: Put into each of two small flasks 10 c.c. of urine and 25 c.c. of distilled water, with a drop of phenolphthalein and a pinch of powdered neutral potassium oxalate. This precipitates calcium and makes the end reaction easier to determine. Then titrate one of the flasks with

0.1 N soda, keeping the other beside it for comparison of tint, and noting the amount required to give the least tinge of pink in the yellow fluid. The result is generally given in the number of c.c. required to neutralize 100 c.c. of the urine to phenolphthalein. (Keep the flasks and their contents for the next experiment.)

It would be better if, as well as this figure, the number of c.c. of 0.1 N acid required to give the methyl orange reaction in 100 c.c. of urine were also given. For it should be clearly understood that the amount of soda used in simply titrating with phenolphthalein is no indication of the acidity properly speaking, the concentration of hydrogen ions, in the urine. Of two urines the one that requires the greater amount of soda to give the phenolphthalein reaction may or may not be the more acid. In one flask dilute 2 c.c. 0.1 N H_2SO_4 to 20 c.c. with water, in the other put 20 c.c. of acid phosphate solution 1.4% (0.1 M). The former has more than 100 times the higher hydrogen ion concentration, is the more intensely acid, but the latter requires about 10 times more soda to give the phenolphthalein reaction, or has the greater acid capacity. Show that this is so.

Or take 100 c.c. of distilled water, add a drop of phenolphthalein and from a graduated pipette or burette 0.1 N. NaOH till a pink colour is obtained. This will be with about 0.1 c.c., that is to say with a concentration of NaOH, and therefore of hydroxyl ion, in the liquid of less than 10^{-4} N.

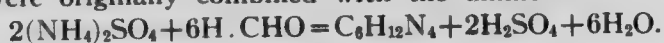
Then take another 100 c.c. of distilled water, a drop of methyl orange and add 0.1 N sulphuric acid similarly till a red colour is obtained, which will be again with about 0.1 c.c. when the concentration of acid and therefore of H ion is less than 10^{-4} N.

Now repeat both these titrations with 100 c.c. of urine instead of the distilled water, several c.c. will be required for each titration; but the urine is not both more alkaline and more acid than the distilled water, it has a higher acid capacity, as any solution of acid and dibasic phosphates has. The same is true of the blood, and in the blood this is one of the mechanisms that is of great importance in helping to preserve the reaction of the blood pretty near a constant value. Acids are necessarily produced in the metabolism of proteins; they are also produced abnormally in carbohydrate starvation and certain intoxications, by CHCl_3 for instance (acetonuria). These acids entering the blood cannot affect the concentration of hydrogen ions to anything like the same extent as they would that of water.

The other protective mechanisms for preserving the normal reaction of the blood are: (1) the excretion by the kidneys of compounds that tend to disturb it; the kidneys being able to remove such substances, in whichever direction the disturbance that they tend to produce might change it, and to excrete urine in which the hydrogen ion concentration is at one time more than 10^{-5} N, at another less than 10^{-8} N.

(2) And secondly the fact that ammonia is constantly being formed in metabolism and that though it is usually converted into the neutral body urea, it is always possible for it to be diverted from this usual course of metabolism and detained for the neutralization of acids that would otherwise affect the reaction of the blood harmfully. In such circumstances the "ammonia coefficient" of the urine (see below) is necessarily higher than normal.

Determination of ammonia in urine. Add 4 c.c. of commercial formalin, that has been neutralized to phenolphthalein, to the neutralized urine and note that the original tint is restored. Now titrate again noting the additional amount of soda required to bring back the pinkish colour. Calculate the amount of ammonia in 10 c.c. and in the total 24 hours' output (1 c.c. of 0.1 N. NaOH = 1.7 mg. NH_3 or 1.4 mg. N as NH_3). The formalin reacts with ammonium salts, forming hexamethylene tetramine (urotropine) and setting the acids free that were originally combined with the ammonia.



For the significance of the ammonium salts in urine see below, the ammonia coefficient.

The following table gives typical figures for the amounts of the different inorganic bases and acids that may be found in the urine passed in 24 hours with their equivalents in terms of normal acid and alkali. Of these the greater part is introduced into the body with the food in the form of neutral salts, nearly all the sodium chloride at any rate and a large part of the phosphates, though little or none of the sulphate.

Na(6 g. in 15 g. NaCl)	equivalent to 250 c.c. normal alkali			
K(3 g. in 3.5 g. K_2O)	"	"	70	"
Ca(0.2 g. in 0.3 g. CaO)	"	"	5	"
Mg(0.3 g. in 0.5 g. MgO)	"	"	12	"
NH_3 (0.7 g.)	"	"	40	"
Total bases		"	377	"

HCl(in 15 g. NaCl)	equivalent to	250 c.c. normal acid
P ₂ O ₅ (2.5 g.)	" "	50* " " "
H ₂ SO ₄ (2.5 g.)	" "	50 " " "

Total acids " " 350 c.c.

With regard to these figures it should be further noted:

(1) That the urine contains about twice as much sodium as potassium, whereas in the body as a whole there is about three times as much potassium as sodium;

(2) That the urine contains more magnesium than calcium, the body as a whole 40 times as much calcium as magnesium; the faeces usually contain more calcium than the urine;

(3) That the urine contains only traces of iron, about 1 mg. in 24°—the faeces usually considerably more, 8 to 10 mg.;

(4) That there is commonly about as much P₂O₅ in the faeces as in the urine, but the ratio is not constant and is altered by the diet and other circumstances, so that the estimation of P₂O₅ in the urine is purposeless unless the faecal output is determined too.

The chlorides are the most abundant of the salts in urine and are commonly given as equivalent to 15 g. of sodium chloride in 24 hours making 1% of this salt. The amount depends on how much is taken with the food; more appears with animal, less with vegetable food. It is diminished in inanition and in fever, especially pneumonia, before the crisis when it increases. Volhard's method of estimating chlorides consists in precipitating with excess of standard silver nitrate solution and titrating the excess of silver in the filtrate with standard thiocyanate solution. The silver solution contains 29.075 g. AgNO₃ in 1 l. and 1 c.c. = 10 mg. NaCl. The thiocyanate solution must be standardized by titrating with it 10 c.c. of AgNO₃ solution to which 5 c.c. of strong nitric acid, a few drops of iron alum solution and water to about 50 c.c. are added; the first sign of permanent red ferric thiocyanate gives the end point. Calculate how much AgNO₃ solution corresponds to 1 c.c. of thiocyanate. Now pipette 10 c.c. of urine into a 100 c.c. measuring flask, add 5 c.c. of strong nitric acid and 20 c.c. of the silver solution. Fill to the mark with water and shake well. Filter about three-quarters through a dry paper into a dry beaker rejecting the first few drops. Pipette off 50 c.c. of the filtrate, add a few drops of iron

*Assuming for the phosphoric acid that a little more than half is present as acid salt, and a little less than half as dibasic salt. Organic acids will account for the neutralization of the excess of bases in the above table.

alum solution and run in thiocyanate from a burette till a permanent red tinge begins to appear: note the amount necessary and calculate the corresponding amount of silver solution: deduct this from 10 c.c. and the result multiplied by 10 gives the number of mg. of NaCl in 5 c.c. of urine. Calculate the output of NaCl in 24°.

Sometimes the addition of nitric acid to urine causes a red colour to appear which makes the end reaction hard to see; in that case a few drops of permanganate will remove the red colouration; any excess of permanganate can be reduced with sugar, and then the titration carried out.

The phosphates in urine correspond to from 2 to 3.5 g. P_2O_5 , according to the nature of the food taken, the nucleoproteins of cells and the phosphoproteins of eggs and milk increasing the output. In the dissolution of tissue too, such as occurs in acute yellow atrophy of the liver, the output is increased. They are estimated by precipitation with standard uranium nitrate solution, excess of which turns coccineal green and gives a brown ppt. with potassium ferrocyanide. Pipette off 50 c.c. of urine into a beaker, add 5 c.c. of 10% sodium acetate in 3% acetic acid and boil. Now add a few drops of coccineal solution and place with a glass rod on a white tile several small drops of ferrocyanide solution. Run the standard uranium solution from a burette into the boiling urine till a green colour appears and then drop by drop till a glass rod dipped in the beaker gives a brown colour with one of the drops of ferrocyanide. The uranium solution is prepared by dissolving 38 g. of uranium nitrate and 3 g. of sodium acetate in 1 litre, titrating it against a standard solution of KH_2PO_4 and diluting it so that 1 c.c. = 5 mg. P_2O_5 . Calculate the excretion in 24 hours.

The sulphates in urine corresponding to from 2 to 3 g. of H_2SO_4 are for the most part (about 90%) inorganic sulphates of sodium and potassium, the rest organic phenol and indoxyl sulphates of bases C_6H_5- or $C_8H_6N-SO_4K$. The total amount varies with the protein combustion in the body, the amount of organic sulphates in some degree with the decomposition of tyrosine and tryptophane by bacteria in the intestine.

The estimation of sulphates cannot be done without the use of an accurate balance.

In the determination of total nitrogen (Kjeldahl) the N of urea, creatinine, uric acid and of all forms is converted into and estimated as NH_3 . Normally 90 to 95% of the N of the food is found in the urine, the rest in the

faeces, the output is equal to the intake (N equilibrium) and varies with it (10-20 g.); it is greater than the intake only when this is insufficient. Loss of nitrogen, consumption of body protein, occurs in inanition and wasting, in fever and hyperthyroidism.

Method: most organic compounds when heated with strong H_2SO_4 are oxidised and give up their nitrogen as NH_3 which is bound by the acid: when excess of soda is added this is set free and can be distilled off and caught in a known excess of standard acid. Then by titrating the acid the amount neutralized by the NH_3 is determined. With a pipette measure 5 c.c. of urine into a flask of resistant glass with round bottom and long neck. Add about 10 c.c. of strong H_2SO_4 and a fragment of copper wire and heat in a draught cupboard till the brown and yellow colour has gone (30-60 mins.). *+ H_2SO_4 as catalyst*

Meanwhile *prepare 0.1 N. H_2SO_4 :* mix 3 c.c. of strong H_2SO_4 with water in a 100 c.c. cylinder and fill up to 100 c.c. Make sure that you have fully 3 c.c. of the acid and that it is thoroughly mixed. Since 1 c.c. of strong H_2SO_4 weighs 1.8 g., 3 c.c. weigh 5.4 g.; and since half the M.W. of H_2SO_4 is 49, 5.4 g. should be when diluted to 100 c.c. a little more than normal strength. Measure out 50 c.c. with a pipette and dilute in a 500 c.c. flask to 500 c.c. To standardize this, take 10 c.c. with a pipette and dilute in a small flask with 20 c.c. water and see how much 0.1 N. NaOH is required to neutralize it to alizarin: if 10 c.c. exactly then it is decinormal, if more calculate how much water must be added to the 490 c.c. left to make it decinormal, if less write your result on the label of the bottle in which you keep it.

Now reckon about how much of this acid is likely to be required to neutralize the NH_3 from 5 c.c. of the urine. If the food contains 100 g. of protein with 16 or 17% of N there will be about 15 g. of N in the urine of 24 hours; and if the volume is 1500 c.c. that will give 1% of N; if it is 500 c.c. 3%. In fever, hyperthyroidism, underfeeding or wasting disease the urine may contain more nitrogen than the food. In 1 c.c. of 0.1 N. NH_3 there is 1.4 mg.

With a pipette measure an excess into the bottle used for receiving the distillate, add some alizarin red and put it ready under the condenser. When the combustion products have cooled down, add water carefully and gradually, cooling the flask under the tap till the flask is just about half full and not more. Mix thoroughly and when it is cool run in under the acid about 40 to 50 c.c. 40% NaOH and at once connect up with the

condenser; then shake round to mix the contents of the flask and light the flame under it. See that water flows through the condenser. If the fluid in the receiver turns colour, at once add 10 c.c. of 0.1 N.H₂SO₄. Distil off $\frac{1}{3}$ of the contents, then disconnect the flask and turn out the flame, wash the end of the condenser tube inside and out into the receiver, and titrate. Each c.c. of 0.1 N. acid neutralized by NH₃ = 1.4 mg. N.

The Ammonia Coefficient Normally the N in the urine is distributed between the different nitrogenous substances approximately as follows (Folin):

I.		II.	
On a full diet, 40 Cal. per kg. and 16 g. N; no purines. Nitrogenous equilibrium.		On a low nitrogen diet, 40 Cal. per kg. and 1 g. N; no purines. 4 g. N excreted.	
Urea.....	87% of total N		60%
Creatinine..	4% of total N		16%
Ammonia..	4% of total N		11%
Uric Acid..	1% of total N		2%
Etc.....	4% of total N		11%

Energy equal.

In the first case more nitrogen is excreted in the form of NH₃ than in the second, but the ammonia coefficient, that is to say, the percentage of the total nitrogen which the ammonia nitrogen forms, is lower. In many pathological conditions the ammonia coefficient may be as high or even much higher than on the low nitrogen diet in health.

Normally about 87% of the total N is excreted as urea and 4 or 5% as NH₃. But the urea is made in the body from NH₃ derived from amino acids, and if acids are formed that cannot be oxidized to CO₂ and so excreted by the lungs, they must be neutralized and excreted by the kidneys, and if there is more of these acids than can be neutralized by the fixed bases that can be spared, then ammonia that would otherwise have been converted into urea is made use of and appears with them in the urine. The acids in question may be H₂SO₄ from the oxidation of excessive amounts of proteid, or β oxybutyric when this fails to be further oxidized. In such cases less of the total nitrogen excreted is in the form of urea and more in that of ammonia and the ratio of the latter to the total nitrogen is more than the normal 4% and may be even more than 40%. Having determined the total N determine next the ammonia coefficient.

The estimation of urea is not practicable in ordinary clinical work. The method commonly in use of estimat-

ing urea with hypobromite really is a rough method rather for estimating total N, as besides urea which is only incompletely converted into N gas, NH_3 , uric acid and creatinine, also more or less incompletely, give up their N as gas. The method is rapid and in spite of its inaccuracies may be useful. 2 c.c. of bromine is added to 25 c.c. of strong caustic soda, the ureometer filled with the solution, and 1 c.c. of urine introduced with a curved pipette. Compare the result with that obtained by Kjeldahl's method. $\text{H}_2\text{N} \cdot \text{CO} \cdot \text{NH}_2 + 3\text{NaBrO} = \text{N}_2 + \text{CO}_2 + 2\text{H}_2\text{O} + 3\text{NaBr}$. The CO_2 is detained by the excess of NaOH.

In the estimation of uric acid remember that it is formed from the purine bases contained in nucleic acid, supplied either by the food or by the cells of the body, and therefore in order to know whether the latter are parting with more or less nucleic acid than normal we must know how much the food is supplying, or better see that the food supplies none by giving eggs and milk, but no meat and especially no liver, thymus or pancreas. Uric acid is excreted in larger amounts in fever and after muscular activity (fits, labour).

The excretion in chronic gout is often low and delayed. Uric acid is precipitated as ammonium salt by $(\text{NH}_4)_2\text{SO}_4$ and NH_3 almost completely.

To 100 c.c. of urine in a dry beaker add ^{exactly} 25 c.c. of a solution of 500 g. of $(\text{NH}_4)_2\text{SO}_4$ and 5 g. of uranium acetate in 60 c.c. of 10% acetic acid and 650 c.c. of water. Mix and let a colloid ppt. settle for $\frac{1}{2}$ an hour; filter through dry paper into a dry 100 c.c. measure and mix 100 c.c. of the filtrate with 5 c.c. of strong ammonia and leave it for 24 hours. Filter and wash the ppt. with 10% ammonium sulphate solution on to the filter and wash on the filter three times. Stir 15 c.c. of strong H_2SO_4 into 100 c.c. of water in a flask, then place the filter and ppt. in the flask, and titrate hot with 0.05 N KMnO_4 till a pink tinge remains throughout the fluid for some seconds. 1 c.c. $\text{KMnO}_4 = 3.75$ mg. uric acid; calculate the result for 100 c.c. of urine and add to it 3 mg. as a correction for the solubility of ammonium urate. Calculate the "Uric Acid coefficient" (Uric acid = $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$).

Standard KMnO_4 solution, 0.05 N, is prepared by dissolving 0.8 g. KMnO_4 (MW. 158) in 500 c.c. water and seeing how much of the solution is required to give a lasting pink tinge in a hot solution containing 10 c.c. of 0.1 N. oxalic acid and some sulphuric acid: if 20 c.c. then the solution is 0.05 N. Since $2\text{KMnO}_4 + 5\text{C}_2\text{H}_2\text{O}_4$

pen for dist.

Folin

In stoppered
Challenmeyer
flask.

in which portion
look place

Uric acid in grams.
per 100 c.c. of 13 Wgt

C.C. + add
H₂SO₄ will react
to the water

$+3\text{H}_2\text{SO}_4 = \text{K}_2\text{SO}_4 + 2\text{MnSO}_4 + 10\text{CO}_2 + 8\text{H}_2\text{O}$, that is to say, 2KMnO_4 supplies in acid solution 5 atoms of bivalent oxygen, twice the molecular weight of KMnO_4 divided by 10 is the number of grams required for 1.L. of normal permanganate solution.

GLYCOSURIA.

Glucose occurs in normal urine only in traces, unless extraordinary amounts of sugar are taken with the food (more than 2 g. per Kg. of body weight at a time). Such alimentary glycosuria occurs after smaller doses in certain people: in diabetes glycosuria occurs if any carbohydrate at all is taken, or in severe cases even when none is taken. In diabetes the urine, besides containing sugar, is usually increased in volume and pale in colour, but nevertheless has a high specific gravity. It is usually also markedly acid, since the nitrogen content is high and therefore the amount of H_2SO_4 too.

In the detection of glucose. (1) Moore's test is useful: mix a test tube nearly full of the urine with a little caustic soda and heat the upper portion: it will become darker than the lower cool portion.

(2) Reduction tests depend like Moore's test on the aldehyde group (neither test is given by cane sugar). Cupric hydrate, formed when NaOH is added to solutions of copper salts, may be held in solution by glucose or cane sugar, by glycerine, tartrates and other substances, and such a solution when heated if no reduction takes place remains clear and blue. If reduction takes place the blue cupric hydrate is converted into yellow or red cuprous hydrate or oxide which is insoluble, unless ammonia is present, with which it gives a colourless solution or purine bases which give a white or grey precipitate with it. If the cupric hydrate is not held in solution by tartrate or other similar substances it turns dark brown on heating, losing water and turning into CuO . Even in the presence of reducing agents if there is more copper than these can reduce, the brown CuO formed may prevent the detection of reducing sugar. In testing for sugar in urine with copper therefore, use cupric hydrate dissolved with tartrate (Fehling's solution) or glycerine (Haines' solution) or boiling these with cane sugar, and remember that the ammonia salts of the urine enable it to hold up a certain variable amount of Cu_2O if formed.

Determine how many drops of urine from a graduated pipette go to 1 c.c. Then boil 2 c.c. of Haines' solution,* and add from this pipette first 2 drops of the urine and boil up again. If the fluid turns red or yellow so that no blue colour can be seen then the urine contains much sugar, more than 2% at any rate. If a blue colour can still be seen then add 3 drops more of the urine and boil again. If now the blue colour is entirely gone then the urine probably contains more than 1% and less than 2% of sugar. If still blue add 5 drops more and boil again. If this gives complete reduction there is probably about 0.5% sugar. If not, repeat finally by adding 10 drops more and again boiling. If as much as 1 c.c. of urine is required to completely remove the blue colour from 2 c.c. of Haines' it is a question whether the reducing power of the urine is greater than it may be normally, and to decide this question may be impossible without experience, care, and the use of other tests as well. Uric acid and creatinine, which will reduce cupric oxide, are usually present in too small amount to cause confusion with glycosuria; but in concentrated urines they may do so, and in doubtful cases Nylander's solution (made like Fehling's solution with bismuth subnitrate instead of copper) may be used, as it is not affected by these substances. (Subnitrate of Bismuth 2 g., Rochelle salt 4 g. dissolved in 100 c.c. of 8% NaOH.) Boil together one part of the solution and 10 of the urine and keep them boiling, best in a boiling bath, for two minutes, then put the tube aside to cool; a black precipitate of reduced bismuth indicates sugar.

The difficulty of doubtful cases is due to the fact that normal urine reduces, often as much as a 0.2% solution of glucose; of this reducing power about $\frac{1}{3}$ is due to actual glucose, $\frac{2}{3}$ to creatinine and uric acid, and $\frac{1}{3}$ to unknown unfermentable substance. In concentrated normal urine the reducing power may be higher still.

(3) *The osazone test* is especially valuable in cases of doubt. Fill the bottom 2 cm. of a test tube with hydrochloride of phenylhydrazine, the next 2 cm. with sodium acetate crystals and just cover with water, warm till dissolved, filter if not clear, add an equal volume of urine and heat in a boiling bath for 30 mins. Cool slowly and examine the bright yellow ppt. obtained if glucose was present, under the microscope.

*Recrystallized copper sulphate 4.0 g. Potassium hydrate 22.5 g. Glycerine 45 c.c. Water to 500 c.c. This solution keeps well, and 1 c.c. requires about 1 mg. of sugar to completely reduce the copper that it contains.

~~5. Methyl~~
(4) *Fermentation test.* Place a piece of glass tubing 1.5 in. long, sealed at one end with the closed end uppermost, in a test tube and cover it with urine: boil for a few minutes and then cool. When cold add yeast; in the course of 24^h gas will collect in the small tube which will be absorbed if the urine be made alkaline with caustic soda. Controls should be done (1) with normal urine, (2) with normal urine to which glucose has been added, to test the yeast, and its autofermentation.

When urine contains more than 1% of glucose it is almost impossible for any doubt to arise. But as the relative proportion of other reducing substances present with the sugar increases, the difficulties increase. Obtain some urine containing about 1% of glucose and compare with the behaviour of this to the above tests that of the same urine diluted with one, four and nine parts of normal urine, and so get familiar with the behaviour of sugar in urine in different small amounts.

For the estimation of glucose in urine prepare Haines-Purdy solution. Obtain copper sulphate in fine crystals by stirring and cooling quickly a hot saturated solution: filter off the crystals and press out the mother liquor and dry in the air without heat (since $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ readily gives up water when heated or over H_2SO_4). Next day when dry weigh out 0.83 g. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, dissolve it in water and wash into a 100 c.c. measuring flask, add 35 c.c. strong ammonia and 4 c.c. of glycerine, then dissolve 2.5 g. of potassium hydrate in water and add that to the mixture and make up to 100 c.c. when cold.

The ammonia is added in order to dissolve Cu_2O as it forms: this solution is colourless and therefore when all the CuO has been reduced to Cu_2O a colourless solution is obtained.

For the estimation 10 c.c. of this solution is used and this requires exactly 10 mg. of glucose to reduce it. There are 10 mg. of glucose in 1 c.c. of a 1% solution, and the measurement of 1 c.c. of urine from a burette, and still more the smaller volume of a stronger solution, cannot be done sufficiently accurately; so the urine is always diluted so as to give a solution containing between 0.1 and 0.2% sugar of which between 10 and 5 c.c. not more nor less, will be required to complete the reduction of 10 c.c. of the copper solution. The amount of dilution necessary is determined by preliminary tests and the method of doing the qualitative copper test described above will with care generally suffice.

Fill with the diluted urine a burette, the point of which passes through the cork in a small flask which has

another tube passing through it connected by tubing to the sink with water running in it. In the flask place 10 c.c. of the Haines-Purdy solution and 30 c.c. of water. Heat the flask and run into the boiling fluid the urine till the blue colour just disappears. The NH_3 and steam displace the air and so prevent reoxidation of Cu_2O . The first estimation gives an idea of the amount of urine required; on repeating it a more exact result can be obtained by means of this information. Calculate the percentage and 24 hours' output of glucose.

ACETONURIA.

Diabetic Urines (Fats)

In starvation, whether due to abstention from food or to vomiting and diarrhoea, and also when though fats and proteins are taken no carbohydrates at all are included in the diet, or when as in severe diabetes any carbohydrate that is taken is excreted as glucose in the urine, acetone CH_3COCH_3 is found in small amounts in the urine and in the breath. It is formed by the splitting off of CO_2 from acetoacetic acid, which takes place in the blood, or even in the bladder, so that this acid accompanies acetone in any but the slightest degrees of acetonuria (more than 0.5 g. daily). In the more severe cases β oxybutyric acid is also found in the urine and then sometimes in large amounts (up to 50 or 100 g.). It is by oxidation of this acid that the acetoacetic acid is supposed to be formed; and the β oxybutyric acid is supposed to be a normal product of the intermediate metabolism of fats and certain amino acids and to appear unburnt in the urine when the oxidation of these substances is incomplete.

Acetone in urine to which 2 or 3 drops of a fresh solution of sodium nitroprusside and some caustic alkali are added, causes a red colour which on acidification with acetic acid turns a dark cherry colour. The red colour given by these reagents in normal urine, containing no acetone, is due to creatinine, and is turned green by acetic acid. Acetone boils at 56°C ., so if 50 c.c. of urine which contains it is distilled, most of the acetone is obtained in the first few c.c. that distil over. It may be detected in the distillate by adding some solution of iodine in potassium iodide and then caustic soda till the yellow colour of the iodine has almost disappeared. If the mixture is heated till nearly boiling and then cooled minute hexagonal crystals of iodoform will appear. Alcohol also gives iodoform under these conditions; but the risk of confusion with alcohol may be avoided by

testing with a drop of tincture of iodine and some ammonia; acetone gives iodoform in this case too; a black ppt. of nitrogen iodide is formed at the same time which gradually disappears leaving the iodoform visible.

Acetoacetic acid gives a brownish purple or bordeaux colour with ferric chloride. The ferric salt first precipitates phosphates, which may be filtered off, and the filtrate with excess of ferric salt shows the colour; or the ferric phosphate may be dissolved by adding more ferric chloride and the colour shown up. On standing it fades slowly, more rapidly if heated: or if the urine is boiled for a few minutes before the ferric solution is added the colour cannot be obtained: this serves to distinguish the acid from other substances which under these conditions too still give a red or violet colour with ferric chloride, such substances as are found in the urine after administration of salicylates, salol, antipyrine, phenacetine or aspirine. If the urine be acidified with hydrochloric acid and shaken with ether, the ether dissolves out the aceto acetic acid and this solution removed and layered on dilute aqueous ferric chloride solution gives to the latter the characteristic colour, while acetic or formic acid in this case imparts a red colour to the ethereal layer.

β oxybutyric acid may be tested for by distilling without a condenser a mixture of equal parts of urine and strong sulphuric acid (sugar should have been fermented off): crotonic acid will crystallize from the distillate. The crystals melt at 72° C.

The estimation of the degree of acetonuria should be done when possible by estimation of the β oxybutyric acid: but it is generally easier to determine the ammonia coefficient and to form in that way an idea of the amount of unburnt organic acid excreted.

PROTEINS IN URINE.

The following proteins may be found in urine and must be carefully distinguished from one another on account of the very different significance which they have:

(1) *Precipitated by acetic acid in the cold*, often traces of a mucin-like substance may be found, probably from the mucous membrane of the urethra or bladder. In larger amounts a precipitate may be obtained, sometimes in fever, in jaundice or after muscular exertions. If unaccompanied by other protein it is of no great import.

(2) *Coagulable proteins*, albumin and globulin, in varying proportions are found in true nephritis and in amyloid disease of the kidney, in various disturbances of the circulation, occlusion of the ureter and after exposure to cold.

Coagulation test. When coagulating proteins by heat see that the reaction is and remains acid, and that there is sufficient salt. Acidify with dilute acetic acid and if necessary add some saturated NaCl solution. If the urine is not clear after adding acetic acid filter. Heat the upper portion of the column of liquid to boiling and note any change in comparison with the lower cool portion. If the urine is not acidified phosphates may be precipitated by boiling.

Nitric acid test. With a pipette very carefully run some urine on to the surface of $\frac{1}{2}$ an inch of nitric acid in a sloping test tube and without disturbing the surface set the tube aside for 2 mins. If albumin is present a white layer of precipitated albumin will lie above the nitric acid: it is seen best by holding the tube against a dark background. In concentrated urine, crystalline urea nitrate or uric acid may appear in the same position. If there is doubt dilute the urine first. Resin acids (copaiba) are also precipitated, but they dissolve in ether.

Hydroferrocyanic acid test. Acidify the urine fairly strongly (2%) with acetic acid and run in a drop of ferrocyanide of potassium: a precipitate is given by albumin. *Picric acid* gives a precipitate with albumin after acetic acid has been added.

(3) *Albumose* occurs in urine in pneumonia when the exudation is being absorbed, also in ulceration of the intestines and accompanying deep seated suppuration. When it accompanies albumin it may be formed from this by the pepsin present in urine, or by bacteria. The urine must therefore be examined fresh. Saturate with ammonium sulphate, boil, cool and filter. Extract the urobilin from the precipitate by washing with alcohol and then dissolve the ppt. with a little boiling water and apply the biuret test to the solution. Urobilin gives the biuret reaction. Albumoses are precipitated by nitric acid, the precipitate dissolves when heated and returns when cooled.

(4) *Bence Jones protein.* The urine in cases of multiple myelomata, made acid and heated, turns milky at 50°, gives a sticky precipitate at 60°, which dissolves clear again at 70° but returns on cooling.

The estimation of proteins in urine for clinical purposes may be conveniently done with Esbach's solution in a special graduated tube. Dissolve 1 g. of picric acid and 1 g. of citric acid in about 80 c.c. of hot water, cool and make up to 100 c.c. in a measuring flask, mix well and label Esbach's solution. Fill the Esbach tube to the mark U with urine, add the solution to the mark R, mix thoroughly and allow the precipitate to settle for 24 hours: read on the scale the number of grammes in a litre and calculate the amount in 24 hours' urine. The most accurate results are between 1 and 4 per mille and the urine should be diluted if necessary to get the reading on this part of the scale.

BLOOD AND BLOOD PIGMENTS IN URINE.

The presence of blood in urine makes the urine red or at least a smoky brown, and red blood corpuscles will be found in the centrifuged sediment. It may be due to inflammation, injury or growth in the urinary tract.

Blood pigment, without corpuscles, may be found in other conditions (poisoning by chlorates, AsH_3 , after severe burns, in periodic haemoglobinuria, etc.).

The guaiacum test for blood and blood pigment. Guaiaconic acid, a component of guaiacum resin, is oxidized by nascent oxygen forming a blue substance. Blood pigment liberates nascent oxygen from hydrogen peroxide; saliva and an enzyme present in pus cells does the same, but not after being boiled. Urine that contains pus gives the guaiacum test, but if boiled can subsequently be tested for blood. Add a drop of fresh guaiacum tincture to some urine in a test tube and float on it one or two c.c. of ozonic ether, a mixture of ether and hydrogen peroxide that has been allowed to stand for a week. A blue colour appears where the two fluids meet. If the reagents are active the test is an exceedingly delicate one.

Heller's test may be used when the above reagents are not at hand. Add caustic soda to the urine and boil; the haemoglobin is destroyed and the haematin split off; the earthy phosphates are precipitated and carry down the haematin, which colours the precipitate brown. In normal urine the phosphates precipitated by alkali come down white. But the phosphates are coloured also when precipitated by alkali in urine containing haematoporphyrine.

Benedine Test.

Spectroscopic tests are the safest and most conclusive. If urine containing blood (and therefore coagulable protein) be boiled so as to coagulate the haemoglobin and then filtered, the precipitate, or equally well the coloured phosphate precipitate obtained in doing Heller's test, pressed dry and boiled in a test tube with a little alcohol acidified with hydrochloric acid will give a reddish-brown solution of acid haematin showing the band in the red of the spectrum. If the filtered alcoholic solution is too dilute or bulky add 2 or 3 c.c. of ether, mix and then add enough water to make the ether separate at the top; a little saturated salt solution will sometimes help: examine the ethereal layer with the spectroscope. The ethereal solution may then be pipetted off into another tube and 2 c.c. of ammonia added; the haematin is converted into alkaline haematin which leaves the ether and goes into the ammonia. Its spectrum shows one band blocking out the orange and yellow between the red and the green, and if to this a little ammonium sulphide is added, or Stokes' fluid (a solution of ferrous sulphate and Rochelle salt with ammonia added) the brown fluid becomes red with reduced alkaline haematin, the spectrum of which (two bands in the green, the left hand one strongest) is very clear even when the other haematin spectra are faint.

The fresh urine will sometimes, if there is enough blood, show the oxyhaemoglobin or methaemoglobin spectrum. But the haematin tests are more delicate as the haematin from 40 or 50 c.c. of urine may if necessary be obtained in solution in 2 or 3 c.c. of ether.

The Haemin test may be done with the coagulated haemoglobin or with the phosphate ppt. obtained in doing Heller's test. A small particle of the ppt. pressed dry, is put on a slide with a small crystal of salt, covered, glacial acetic acid run under the cover and either left at the room temperature for some time or warmed over a flame without allowing the acid to boil: as it evaporates fresh acid must be added. The crystals are visible when magnified 200 or 300 times and are brown and rhombic in shape.

Haematoporphyrin occurs in traces in the urine normally, and in significant amounts in cases of suppurational poisoning and rarely in certain other pathological conditions. It is carried down with the phosphates by baryta mixture or soda and can be extracted from the precipitate with acid alcohol and recognized by its spectrum. Make a drawing of the spectrum.

BILE AND OTHER PIGMENTS IN URINE.

When the bile ducts are obstructed bile enters the blood and appears in the urine. The demonstration of bile acids in the urine stained with bile pigment cannot usually be done by means of Pettenkofer's reaction unless they are separated and extracted first. But their presence lowers the surface tension of the urine for flour of sulphur, so that this sinks instead of floating as it does on normal urine: show this, using normal urine for a control (Hay's test). The presence of bile pigments is however easier to detect.

Gmelin's test for bile pigment may be done by carefully floating the urine on nitric acid and obtaining a series of coloured rings, green, blue, violet, red and yellow from above downwards: or the edges of a drop of urine and a drop of nitric acid may be allowed to meet on a porcelain plate, or again a filter through which the urine has passed two or three times may be partially dried with dry paper and touched with a drop of the acid; the outermost of the rings of colour should be green, and the red on the inside edge should be clearly seen. It is well to practise recognizing the test in the first way given, as the routine examination of urine for albumin often betrays the presence of bile.

Huppert's test is useful when the urine is dark or contains much indican. Precipitate the calcium salt of bile pigments by adding calcium chloride and ammonia or soda. Filter off the precipitate and heat it with alcohol acidified with HCl, the alcohol turns green if any bile pigment is present.

The iodine test is done by acidifying the urine with acetic acid and floating carefully on it some 1% alcoholic solution of iodine (tincture diluted nine times); an emerald green ring is seen where the fluids meet.

Urobiline is formed by the reducing action of intestinal bacteria from bile pigments: much of it is discharged with the faeces: some is found in urine which has been exposed to the light, and this is believed to have been absorbed from the intestine and excreted by the kidneys as a closely related colourless chromogen from which it is reformed in the light. The amount in urine is usually small, but considerably increased with intestinal paresis, after jaundice or internal haemorrhage, in scurvy, and sometimes in fever and pernicious anaemia. The largest amounts are found in "acholuric jaundice". The urine is then usually dark from other accompanying pigments. The spectrum of urobiline may be seen in the urine to which HCl is added, a band in the green near the blue,

if strong, absorbing all the blue. If the urine is made strongly alkaline with ammonia and a few drops of 10% solution of zinc chloride added a green fluorescence is observed. The pigment can be extracted with amyl-alcohol. For the preparation of urobiline from faeces, and also for Ehrlich's test for urobilinogen, see below, p.31.

Indican is the name given to the colourless sulphuric acid ester of indoxyl which occurs as a salt in the urine. The indoxyl is formed by oxidation in the body of indol absorbed from the intestine; the indol being there split off from tryptophane by bacteria. The amount of indican in the urine varies therefore with the amount of bacterial decomposition of tryptophane, and inversely with the activity of intestinal movements, especially in the small intestine. It is similarly increased in gangrene of the lung and putrid bronchitis.

Indican can be converted into indigo by oxidizing agents and the amount estimated by the intensity of the colour of its solution in chloroform. To 5 c.c. of urine in a small flask add 5 c.c. of Obermayer's reagent (2 g. of ferric chloride dissolved in 1 l. of strong HCl) and 5 c.c. of chloroform measured with a pipette. Shake gently every few minutes for half an hour and then decant off the acid urine, carefully leaving the chloroform. Dry this by adding one or two grams of anhydrous sodium sulphate and pour off the blue chloroform into a narrow test tube and cork it. Into another tube of the same diameter put 2 c.c. of the Haines qualitative solution for sugar testing. If the chloroform solution is equally dark blue, it is said to have the standard value of 100. Usually it is much paler: in that case run in water from a burette till the diluted Haines solution has the same depth of colour, and from the amount used reckon the fraction of the standard value, e.g., if 5 c.c. is required $2 : 2+5 = x : 100$. Fair comparative results can thus be obtained.

Other oxidizing agents convert indican into indigo but are apt to carry the oxidation on to colourless stages. Indigo red isomeric with indigo blue is also formed in acidified urine under certain conditions.

Ehrlich's diazo reaction, 10 c.c. of a solution of 0.5 g. sulphanilic acid and 5 c.c. hydrochloric acid in 100 c.c. of water with a few drops of a 0.5% solution of sodium nitrite gives diazo benzene sulphonate: $\text{HSO}_2\cdot\text{C}_6\text{H}_4\cdot\text{NH}_2 + \text{NaNO}_2 + 2\text{HCl} = \text{HSO}_2\cdot\text{C}_6\text{H}_4\cdot\text{N} : \text{NCl} + \text{NaCl} + 2\text{H}_2\text{O}$. This shaken with an equal vol. of urine till a foam is produced and then at once treated with excess of ammonia, sometimes gives a red colour in the foam and fluid. What

the substance that causes this reaction is, is not known: but it is present in many febrile conditions: most constantly in measles, typhoid fever and advanced phthisis; often in scarlet fever, pneumonia and erysipelas; seldom in diphtheria, rheumatism or meningitis, and occasionally in non-febrile diseases. A similar colour is obtained after taking certain drugs (opium, creasote, etc.).

Methylene blue (0.1% in water) gives a vivid green colour with the urine in typhoid fever (bile pigment).

Black or dark colouration of the urine occurs in certain rare cases of melanotic sarcoma, of ochronosis in which the cartilages are also found to be stained, and in alcaptonuria. In this last abnormality which is not incompatible with health, tyrosine and phenylalanine instead of being completely oxidized are excreted as alcapnone, 1.4 dioxypyphenylacetic acid, which is quickly oxidized to a black substance on exposure to the air.

Certain common drugs are excreted in the form of compounds that may cause confusion in the analysis of urine.

Rhubarb and senna excreted as chrysophanic acid, and santonin, cause a greenish yellow colour that turns red with alkali.

Resins as copaiba cause the excretion of the resin acids that are pptd. as albumin is by nitric acid, but unlike albumin also by hydrochloric acid. The ppt. is, again unlike albumin, soluble in alcohol or ether. The urine of patients taking iodides gives a brown colour with nitric acid due to the liberation of iodine which can be taken up with chloroform giving a pink solution. Dissolve a crystal of potassium iodide in some urine and observe these reactions.

Salicylic acid is excreted after taking this substance, salol and aspirin; and it gives with ferric chloride a colour like that given by aceto-acetic acid. Dissolve a trace of salicylic acid in some urine and observe that the urine may be boiled for some minutes and still gives the reaction.

URINARY SEDIMENTS AND CALCULI.

The presence of a precipitate of phosphates or of urates in urine must never be taken to mean that phosphates or urates are being excreted in excess. Any alkaline or decomposing urine has a precipitate of phosphates, any concentrated acid urine one of urates. The nature of a urinary sediment is therefore intimately dependent on all that has been learnt about the reaction and concentration of the urine.

The deposits in alkaline urine are mainly amorphous. (1) Earthy phosphates generally white and granular, always soluble in acetic acid; (2) calcium carbonate granules which dissolve in acetic acid giving off gas; (3) acid ammonium urate spherules with adherent crystalline spicules, "hedgehog spines," often pigmented. The characteristic crystalline components of the sediment in alkaline urine are the large colourless prisms ("coffin lids") of the triple phosphate of ammonium and magnesium. They may be sometimes seen glistening to the naked eye.

In acid urine an amorphous deposit of urates is common, coloured orange or pink with uroerythrin ("brick dust"). It dissolves on warming the urine or on adding alkali. Collect a little of the sediment and test for uric acid with the murexide test, heating it with a drop of nitric acid till a dry orange residue is obtained which is turned crimson by ammonia vapour and purple by caustic potash. Calcium oxalate occurs in the form of small dumb bells soluble in HCl, but not in acetic acid, and also in the crystalline form of octahedra, looking under the microscope like square envelopes. Uric acid crystals are usually visible to the naked eye and conspicuous for their reddish brown colour ("cayenne pepper grains"). Under the microscope their colour appears yellowish brown and the shape is characteristic, "hetstones," with two parallel faces and edges curving convexly to a point at each end, or composite clusters of such units. The distance between the two parallel faces may vary considerably and so give rise to very different appearances. Small triple phosphate crystals may be found in urine which is slightly acid.

Rare pathological crystalline forms in urinary sediments are: *tyrosine*, sheaves of fine colourless needles, soluble in ammonia, and *leucine*, small globular forms with a dark centre, resembling oil drops, also soluble in ammonia. These when found are found together; they are almost pathognomonic for acute yellow atrophy of the liver.

Cystine in hexagonal plates occurs in the urine of certain individuals or families who are otherwise healthy, unless the cystine forms calculi. It is soluble in ammonia.

In examining a *urinary calculus* it, or part of it, should be powdered and treated with hot dilute hydrochloric acid as long as any of it dissolves, and the solution allowed to cool and filtered: phosphate and oxalate would be in solution. Any part that does not dissolve

in dilute hydrochloric acid should be tested by the murexide test for uric acid.

The filtered acid solution is then made alkaline with ammonia; any phosphate or oxalate present will be precipitated; but if the fluid is now made acid with acetic acid, if the precipitate was phosphate it will dissolve, if oxalate it will not. Filter off any that does not dissolve and test the filtrate for phosphoric acid with nitric acid and ammonium molybdate.

In this way the commonest constituents of urinary calculi are easily detected and distinguished.

Another way of investigating a stone is to heat some of the powder on platinum foil. If the bulk of it burns away it may be uric acid or ammonium urate—in this latter case hydrochloric acid will remove ammonia (easily detected on adding soda to the filtered fluid) and leave uric acid undissolved, which can be recognized by the murexide test. In rare cases a stone that burns completely may be cystine or even xanthine; the former of these dissolves in alkalies, ammonia or mineral acid, not in acetic acid—the latter is only slightly soluble in hydrochloric acid but does not give the murexide test.

If the bulk of the powder does not burn away (there is almost always some organic matter in any calculus), but the ash moistened with hydrochloric acid effervesces, calcium oxalate was present and has now been converted into carbonate in the flame: if it does not effervesce it is phosphate; if triple phosphate the original powder will give off ammonia when heated with soda; if simply phosphate of calcium and magnesium, of course not.

THE CHEMICAL EXAMINATION OF THE FUNCTIONS OF THE STOMACH.

Test meals, as usually given, are composed of a little dry bread or toast, about 40 g., and some tea without milk or sugar, about 400 c.c., taken first thing in the morning. One hour later this is removed by means of the stomach tube (Ewald). Normally from about 50 to 100 c.c. of fluid is then obtained: if much more than this, e.g., 200 c.c., containing many solid particles, can be withdrawn, defective motility is suspected, and if in addition the fluid is strongly acid then hypersecretion is probable. The fluid is filtered, tested with litmus, and if acid, as it almost always is, then with indicators for *free hydrochloric acid*, that is to say, indicators for a relatively high concentration of hydrogen ions such as is given in fluids of this nature only by the highly ionized acid hydrochloric acid. The indicator most commonly used is Töpfer's reagent (dimethyl amino azobenzene 0.5% solution in alcohol); but congo paper may be used or tropaeolin oo (a trade name for a dye) or Günzberg's reagent (phloroglucin 2 g., vanillin 1 g., 80% alcohol 100 c.c.). In using this last 2 or 3 drops of the stomach contents and 1 drop of the reagent are evaporated carefully in a porcelain dish; a crimson colour is given by free hydrochloric acid.

Strong hydrochloric acid contains about 35% of the acid, is therefore about 10 N, and must be diluted 100 times to give a solution of about the concentration of normal gastric juice. Dilute 1 c.c. of strong HCl with 100 c.c. of water, and similarly 1 c.c. of 60% acetic (10 N), and also 1 c.c. of concentrated lactic acid (also about 10 N), and test these diluted acids with the indicators mentioned. Now take 1 c.c. of each of these diluted acids and dilute it 10, 50, 100 or 200 times till the dilution is reached at which it no longer reacts to Töpfer's reagent and compare the result in the case of the different acids (using of course distilled water for the dilutions). The other indicators mentioned above may be tried at the same time.

Normally gastric juice, containing free hydrochloric acid equivalent to about 0.1 N or a little more, is secreted after the test meal and mixed with the fluid and sus-

pendent solids—some of the fluid is passed on to the duodenum and some of the protein in the bread digested, so that when the contents are removed there should be obtained a diluted gastric juice containing some free HCl and some HCl combined with protein or the digestion products of protein. These combinations are acid to phenolphthalein, but are not sufficiently ionized to give a concentration of hydrogen ion such as is necessary to give a reaction with, for instance, Töpfer's reagent, whereas of course hydrochloric acid itself even in the dilution of 0.001 N, at any rate, one part of gastric juice, that is to say, in 100 of water, reacts distinctly to this indicator. If then 100 c.c. of stomach contents still react acid to Töpfer's reagent on adding 0.1 N. soda until 30 c.c. of the soda has been added, there must be at least the equivalent of 30 c.c. of 0.1 N. HCl free in 100 c.c. of the fluid, or about 30 c.c. of gastric juice over and above that which is combined with proteins or their derivatives. The titration of stomach contents is therefore usually done thus: 10 c.c. of the filtered fluid diluted with 25 c.c. of water is titrated with Töpfer's reagent and 0.1 N. NaOH till the red colour just fades away. It is customary to multiply the number of c.c. used by 10 and give the result as so many c.c. of 0.1 N. *free hydrochloric acid* in 100 c.c. of contents. A drop of phenolphthalein is then added to the same fluid in the same flask and more soda run in till the fluid begins to be pink from the phenolphthalein reaction. The amount of soda used in this second titration corresponds to the hydrochloric acid which has gone into combination with proteins or their cleavage products, together with traces of lactic acid, acid phosphates or other weakly acid substances present in the secretion. The result is generally given as *total acidity*, that is to say, the total amount of soda used in the two titrations multiplied by 10 giving the equivalent of all the acids in 100 c.c. of the fluid. Normally the total acidity is usually about 60 c.c. and the free hydrochloric acid about 30 c.c., for 100 c.c. of the filtered contents.

The disorders in which no free HCl is found after a test meal are primarily carcinoma of the stomach, more especially of the body of the stomach, less commonly if the pylorus is the only part affected unless this has led to dilatation and pernicious anaemia.

In cases in which no free hydrochloric acid is present the extent of this deficiency may be determined similarly by titrating with 0.1 N. HCl, and the result expressed in the same way only with a minus sign prefixed.

The amount of HCl may be diminished in many conditions besides those in which it is usually absent: it is increased in many conditions too but especially in cases of simple ulceration, more especially of the pyloric portion of stomach or adjacent part of duodenum. Even when there is no free HCl the reaction is usually acid, and in this case will probably be mainly due to organic acids formed by microorganisms acting upon the foods, especially the carbohydrates. In this way lactic acid and volatile fatty acids, principally acetic and butyric acids, are produced: these acids are most abundant when the motility of the stomach is impaired.

Lactic acid is tested for with Uffelmann's reagent: to half a test tube full of 1% carbolic acid add 1 drop of 5% ferric chloride solution and dilute the purple solution till it is transparent. A few drops of the fluid suspected of containing lactic acid is then added, and if lactic acid is present the blue colour gives place to a distinct yellow. It is best to extract the lactic acid from the gastric fluid by means of ether, pipette off the ether, evaporate it with a little water and test for the acid in the water.

A more delicate test for lactic acid is obtained if a drop or two of the solution of the ethereal extract is added to 5 c.c. of strong sulphuric acid with 1 drop of saturated copper sulphate solution in a test tube and heated in a boiling water bath for 2 minutes, then well cooled and treated with a drop of 1% alcoholic solution of thiophene. Lactic acid gives a bright cherry red.

The volatile fatty acids are often easily recognized by their sour rancid smell, but should be tested for by shaking 5 c.c. of the filtered fluid with 5 c.c. of ether, pipetting off the ether and evaporating it in a dish after adding 2 c.c. of water. Neutralize with 1% NaOH and add a drop of dilute ferri chloride: if acetic acid is present the red colour of ferric acetate will appear, and on boiling a brown ppt. of the basic salt.

Butyric acid has the smell of rancid butter, and when heated with a drop of alcohol, and strong sulphuric acid, smells of pineapples.

The *peptic activity* of the stomach is less often diminished than the hydrochloric acid. But the absence of both, achylia, is proportionally serious. The peptic activity may be roughly estimated as follows: into each of five tubes place 10 c.c. of a solution of 1 g. of caseinogen in 1 l. of 0.3% HCl, and add to the first 0.1 c.c. of the gastric fluid, to the second 0.2 c.c. and so on. Keep the tubes in a bath at 40° C. for 15 mins. Now

add to each tube a drop of phenolphthalein and NaOH till the pink colour appears, thus stopping at once any further peptic action. Now carefully neutralize with dilute acetic acid. This will precipitate any unchanged casein, but not the products of the action of pepsin. The unit of peptic activity being taken as the amount necessary to completely change 10 mg. of caseinogen, under the conditions described, the experiment makes it possible to give the number of units in 1 c.c. of the gastric fluid.

The spontaneously vomited contents of the stomach may be examined in the same way as the fluid removed after a test meal: but the results are for purely clinical purposes, generally not so easy to interpret nor so instructive. Blood in the vomited matter may, if the result of a recent haemorrhage, be recognizable by its unaltered colour: but if not quite recent the haemoglobin will have been decomposed and haematine split off: material resembling coffee grounds in vomit should be examined for this pigment as described below under the examination of the contents removed after a test meal.

The contents removed after a test meal should be examined for blood in all cases.

THE EXAMINATION OF FAECES

in clinical work is for the most part not of a chemical nature. Test diets are given containing milk, bread, oatmeal, potatoes and lightly cooked meat, and the macroscopic and microscopic inspection of the faecal matter furnishes the most important information with regard to the functions of the alimentary canal.

Chemical examination however is necessary often for the detection of blood pigment, bile pigment or urobilin. Normally if no meat is taken no blood pigment is to be found, and bile pigments do not normally appear below the ileo-colic valve except in traces, being reduced to urobilin almost completely in the colon.

Urobilin is detected by Schmidt's test: a small portion of the faecal matter rubbed to a paste with water is treated with saturated mercuric chloride solution: after some hours normal faeces containing urobilin are coloured red, those containing bile pigment are coloured green and this latter colouration, even if only visible under the microscope, is pathological. *Urobilin is absent only in complete obstruction of the bile ducts: bilirubin present only if the contents pass too rapidly through the large intestine or, when abundant, through the small intestine too.*

When urobilin is not found in the faeces, the absence of urobilin and of urobilinogen from the urine should be established by means of Ehrlich's test with dimethylaminobenzaldehyde which in its most delicate form is done thus: 20 c.c. of urine are acidified with tartaric acid, shaken with 10 c.c. of ether, the ether separated, washed with a little water and then treated with a trace of the reagent as dry powder and a few drops of strong hydrochloric acid: a red watery layer separates if urobilinogen is present and shows with the spectroscope a band in the green near the orange. The urobilin band is often seen too, unless the urine is quite fresh.

A simpler method of testing this may generally suffice, which is to add to the urine in a test tube a few drops of a 2% solution of the reagent in 15% hydrochloric acid and then warm. Normal urine will give a red colour; in some abnormal conditions an intense red colour is obtained even without heating; but if no urobilinogen is present the colour will not be obtained at all. The test cannot be used with faeces or extracts of faeces because indol gives a similar reaction and is generally present in faeces.

Urobilin may be prepared in quantity by stirring faeces up with water, filtering, saturating the filtrate with ammonium sulphate after acidifying, and extracting the filtered ppt. with alcohol which takes up the urobilin. The brown solution on dilution becomes a pinkish yellow and shows a distinct band between the green and blue of the spectrum; with a drop of weak ammonia and some zinc acetate a characteristic fluorescence is to be seen.

Blood pigment is always present in small amount when meat is taken, but otherwise only in disease. It may be recognizable by its red colour and then, if only on the surface of the stool, comes from the neighbourhood of the anus or rectum, if mixed with the mass, from the colon, or rarely, as occasionally in typhoid fever, from the small intestine. Generally if it comes from the small intestine or stomach the colour is altered to dark brown or black, and the tarry altered blood permeates the whole mass. In smaller amounts, "occult blood," it can be recognized only by chemical tests. The faecal matter is rubbed to a paste with water and glacial acetic acid in equal quantities. Ether is added and gently mixed by inversion several times: the ether dissolves out acid haematin which may be recognized by the spectroscope, or if too dilute the ethereal solution may be pipetted off and treated with tincture of guaiacum and ozonic ether, traces of haematin giving a blue colour, or with a saturated alcoholic solution of benzidine and a little 3% hydrogen peroxide an intense green. In the case of fatty stools it is necessary before heating the material with acetic acid to remove most of the fat with ether. In using the spectroscopic test acid haematin should be converted into alkaline haematin with alcoholic potash and then reduced with ammonium sulphide to haemochromogen and all three spectra observed.

The reaction of faeces may vary from weakly acid to weakly alkaline and with but little significance at any rate in adults. Strong acid or alkaline reactions in the faeces of adults, and weaker ones in those of babies are pathological. In the early stages of digestive disturbances the reaction tends to be too acid from fermentation of carbohydrates, at their height alkaline and foul from decomposition of proteins.

ENERGY NEEDS OF THE BODY.

Food is required by men to enable them to maintain the body temperature at a level higher than that of their surroundings, and to do any external work that they may have to do. Food is composed of substances that by their chemical constitution contain energy, which on oxidation of these substances is set free, transformed into work and heat, that is to say, into other forms of energy. Much of the chemical energy that is transformed in the first instance into work in the body, *e.g.*, the work of the heart and of the respiratory muscles, is gradually transformed again, by friction, etc., into heat before this energy leaves the body; some of it, however, is transformed into external work, as when a man loads a waggon with coal, for instance.

Clearly then the amount of food required to meet our daily needs will depend upon the amount of heat we give off, or lose, and also on the amount of external work done.

This amount of food is most conveniently measured in heat equivalents or Calories, and is determined for any individual by the conditions of his life. An adult leading a life of average activity in health and in a temperate climate requires *40 Calories for every kg. of body weight in 24°*. An adult lying in bed at rest, to maintain weight and temperature, requires about $\frac{1}{2}$ of this or 32 Calories per kg.

Since severe muscular exertions increase the rate of oxidation in the body, as measured by CO_2 output and O_2 consumption, and as compared with the rate during perfect rest, sometimes as much as tenfold, it is clear that a man doing hard physical labour for several hours out of the 24 may require to have food the heat equivalent of which is much more than 40 Calories per kg. per 24°: as a matter of fact the requirements of a man may rise to 60 or even 70 Calories per kg. per 24° under such conditions.

But for patients at rest in bed 32 Calories per kg. is as a rule sufficient, that is unless assimilation is imperfect or there are abnormal wasting processes at work, as in typhoid fever.

These figures are for adults. In the case of children with smaller bodies the surface from which heat is con-

tinually lost is greater in proportion to their weight than in the case of adults. The number of Calories that must be supplied to children reckoned per kg. of body weight is therefore necessarily greater.

If the number of Calories needed by a man be reckoned per unit of body surface instead of per kg. of body weight, a figure will be obtained which clearly should be available for calculating the needs of children, if the rate of heat loss is the only factor that determines the greater need for food in children.

The surface of the body bears a constant relation to the body weight, and if this latter in grammes be represented by the letter W , then S the surface in cm^2 is

$$S = 12.3 \times W^{\frac{1}{3}}$$

Thus if a man's weight is 70,000 g.

$$\log. 70,000 = 4.8451$$

$$\text{and } \log. 70,000^2 = 9.6902$$

$$\text{and } \log. 70,000^{\frac{1}{3}} = 3.2301 = \log. 1698$$

$$\text{and } \therefore S = 12.3 \times 1698 \text{ cm}^2 = 20,885 \text{ cm}^2 \text{ or about } 2.1 \text{ m}^2.$$

Reckoning his needs of food at 40 Cal. per kg. or 2800 Cals. this will give the number of Calories per m^2 of body

surface as $\frac{2800}{2.1}$ or 1333: and therefore reckoning his

needs at another period of rest in bed, at 30 Cal. per kg. we get 1000 Cal. per m^2 of body surface.

We may say therefore that a man's needs, as determined by body surface, vary from 1000 Cal. per m^2 when at rest, to 2000 Cal. per m^2 per 24° when doing hard physical work.

Using this figure for calculating the needs of a child weighing 3.5 kg. The surface of the child's body is $12.3 \times 3500^{\frac{1}{3}}$

$$\log. 3500 = 3.5441$$

$$\therefore \log. 3500^2 = 7.0882$$

and $\log. 3500^{\frac{1}{3}} = 2.3627 = \log. 230.5$. So the surface of the child's body is $230.5 \times 12.3 = 2835 \text{ cm}^2$ or 0.28 m^2 , and the child's needs as determined by its body surface should lie between 280 Cal. when at rest, and 560 Cal. when doing hard physical work, which in a child of that age means crying and nothing else. Now working back to the relation of these needs to its body's weight we get that a child of 3.5 kg. needing from 280 Cal. to 560 Cal. per 24 hours needs $280/3.5$, i.e., 80 Cal. per kg. or if crying continually, perhaps even double this amount.

That is, that a baby of 7 or 8 lbs., on account of the relatively large area of its body surface, will need 80 Cal. per kg. per 24° under conditions in which a man needs

only 30, and under conditions such as those under which a man needs 40 Cal. per kg., when leading a life of normal activity, and crying only a moderate amount it will need more than 100 Cal. per kg.

But this is all based on the assumption that the greater needs of children for food are determined solely by their smaller size and consequent greater surface and greater rate of heat loss. But a child requires food not merely for maintaining body temperature and the performance of muscular work; some of the energy of its food should be retained in its body in the form of new tissues produced by the growth of the body. How much has to be allowed for growth can only be determined by experimenting on adults and children of different ages.

Measurement of the oxygen consumption was used by M. J. and Falk for this purpose; they found:

				Oxygen used per m ² and hr.
From 2 to 7 yrs.	of age	from 8.5 to 9.5 litres		
10 to 20	" "	" 7.5 to 8.5		
20 to 60	" "	" 7.0 to 7.5		
60 to 80	" "	" 6.0 to 7.0		

These figures were obtained under conditions, before breakfast and in bed, under which the difference that probably exists between the activity of boys and girls does not appear.

Camerer obtained similar results by determining the value of the food of infants and children at different ages.

	Body weight in kg.	Cal. per kg. per 24°.	Cal. per m ² per 24°.
Boys and Girls 4 to 7 years	13 to 18	69 to 77	1460 to 1680
Boys and Girls 7 to 10 years	20 to 24	59 to 62	1390 to 1440
Boys and Girls 11 to 18 years	32 to 59	33 to 52	1200 to 1400
Man.....	70	32	1070
Woman.....	56	32	1000

In infancy the following figures are given by Camerer:

Age.	Weight.	Cal. per kg.	Cal. per m ² .
1 wk.	3 kg.	70	800
10 wks.	5 kg.	100	1400

During the first week therefore it appears that the food per surface unit is at a lower rate than in an adult at rest (1000 Cal. per m²) and it is common for there to be no gain or even a loss in weight during the first few days of life. But after that, as the child begins to grow, the food consumption per surface unit is considerably higher than it would be in a man in bed and sleeping for more than half the day. Even 40 Cal. per kg. in a man

of 70 kg., which is enough for a very active life, though not for hard manual labour, only gives as we saw 1333 Cal. per m². But on the other hand it does not appear that a healthy growing baby requires more food per unit of surface area than healthy growing children up to the age of 10 years; in fact, rather the healthy active child of 5 or 6 seems to require more than the baby: although the baby grows faster, it is less active, unless it cries much.

A normal infant 10 wks. old weighing 5 kg. and gaining 25 g. a day

A. *Takes as Food*

800 g. human milk

i.e., Protein 10 g. = 40 Cal.

Fat 28 g. = 260 Cal.

Carbohydrate . . 65 g. = 220 Cal.

520 Cal

Ash 1.4

B. *Excretes*

520 c.c. urine with 2.5 g. organic solids } = 40 Cal.

20 g. faeces with 3.6 g. organic solids } = 40 Cal.

C. *Calorie Balance*

Excreted 40 Cal.

Given off as heat . . . 430 Cal. Intake as above:

Retained as tissue . . . 50 Cal. 520 Cal.

520 Cal.

COMPOSITION AND FOOD VALUE OF COMMON ARTICLES OF FOOD.

1 oz. of:	Yields Cals.	Percentage Composition				
		Protein (= N × 6.25)	Fat	Carbo- hydrate	Water.	Ash.
Milk	(17 to) 20	3.3	3.0 to 4.0	5.0	87	0.7
Bread	75	9.5	1.2	53	35	1.2
Butter	225	1.0	85		71	3.0
Eggs (shelled) = 1½ to 2 oz. each	1 egg = 60 to 80	13	12		73	1.0
Beef (acc. to amt. of fat)	40 (to 100)	20 (to 15)	5 (to 42)	1	70 (to 40)	1.0
Whitefish	44	23	6.5		70	1.6
Cheese (Cheddar or Gruyere)	120	27	35	1	32	5.0
Potatoes (boiled)	30	2.5		21	75	1.0
Peas (green boiled)	34	7.0	3.0	14.5	74	1.5

Note that 1 oz. = about 28.5 g.; that the calorie value of 1 oz. butter = 3 oz. bread; and that of 1 egg = $\frac{1}{2}$ oz. cheese = 1 oz. bread = 2 oz. meat = 4 oz. milk = about 80 Cal.

Most kinds of meat (including liver, kidney, sweetbread) and poultry are similar in food value to beef, containing 20 % of protein in the lean portions.

Fowls contain much more fat (16 to 20%) than chicken (2 to 3%); goose up to and more than 30%; turkey 20%; the percentage of protein tends to diminish when that of the fat is high except in turkey (22 to 25%).

Fish vary much in composition:

	Cal.	Protein %	Fat %	Carbohydrate %	Water %	Ash %
1 oz. Salt Cod	= 25	25	0	—	53	23
1 oz. Fresh Cod	= 20	16	0.3	—	82	1.2
1 oz. Halibut	= 35	19	5	—	75	1.0
1 oz. Herring	= 40	19.5	7	—	72	1.5
1 oz. Salmon	= 66	18	18	—	63	1.0

Oatmeal porridge (1 oz. = about 18 Cal.) contains 80 to 85% of water, 2.5 to 3% of protein, 11 to 12% of carbohydrates.

Boiled rice (1 oz. = about 32 cal.) contains about 72% of water, 2.5 to 3% of protein, but 25% of starch.

For these and other data on the composition of food materials, see Atwater and Bryant, U. S. Depart. of Agriculture, Bulletin No. 28.

Crumble 10 g. of bread, weighed to the second decimal place and let the crumbs dry, and then powder them in a mortar without loss till quite fine: dry in an oven at 104° C. till there is no further loss of water. *Determine the water content.*

Take about 0.2 g. weighed to the third decimal place and determine the nitrogen by Kjeldahl's method, and calculate the percentage of protein, using the factor, protein = $N \times 6.25$.

Put about 1 g., weighed accurately, into a 200 c.c. flask; mix about 60 c.c. of water and 1 c.c. of strong sulphuric acid and pour it on to the flask; fit the flask with a condenser and boil gently on a gauze for 2 hours. Filter into a 100 c.c. measuring flask through a small filter, wash with water and make up to the mark. Mix and estimate the sugar; calculate the amount of starch (= sugar - 10%).

Take 5 g. of lean meat, cut off a piece weighing about 1 g., weighed to third decimal, put it in a Kjeldahl flask and determine the N. Cut the rest as fine as possible and weigh it in a watch glass or flat dish. Dry it in an oven at 104° to constant weight and determine the percentage of water. Calculate the percentage of protein in the fresh meat.

INSPECTED
KANSAS INSPECTION & TESTING LABORATORIES, LANSING

By _____